

Application No.: 10/648,152  
Docket No.: CL2123 US NA

Page 2

### **Listing of the Claims**

This listing of claims will replace all prior versions, and listings of claims in the application

1. (original) An isolated nucleic acid molecule encoding an  $\alpha(1,6)$ -linked glucose oligosaccharide hydrolyzing polypeptide selected from the group consisting of:
  - (a) an isolated nucleic acid molecule encoding the amino acid sequence SEQ ID NOs:2, 4, or 6;
  - (b) a nucleic acid molecule that hybridizes with (a) under the following hybridization conditions: 0.1X SSC, 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS; and
  - (c) a nucleic acid molecule that is complementary to (a) or (b).
2. (original) The isolated nucleic acid molecule of Claim 1 selected from the group of nucleic acid molecules consisting of SEQ ID NOs:1, 3, and 5.
3. (withdrawn) A polypeptide encoded by the isolated nucleic acid molecule of Claim 1.
4. (withdrawn) The polypeptide of Claim 3 selected from the group consisting of SEQ ID NOs:2, 4, and 6.
5. (original) An isolated nucleic acid molecule encoding an  $\alpha(1,6)$ -linked glucose oligosaccharide hydrolyzing polypeptide selected from the group consisting of:
  - (a) an isolated nucleic acid molecule encoding a chimeric protein comprised of a signal peptide operably linked to an  $\alpha(1,6)$ -linked glucose oligosaccharide hydrolyzing polypeptide;
  - (b) a nucleic acid molecule that hybridizes with (a) under the following hybridization conditions: 0.1X SSC, 0.1% SDS, 65 °C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS; and
  - (c) a nucleic acid molecule that is complementary to (a) or (b).
6. (original) The isolated nucleic acid molecule of Claim 5, wherein the signal peptide is SEQ ID NO:24 or SEQ ID NO:25.
7. (original) The isolated nucleic acid molecule of Claim 5, wherein the  $\alpha(1,6)$ -linked glucose oligosaccharide hydrolyzing polypeptide is SEQ ID NOs:2, 4, 6, 17, or 31.
8. (original) The isolated nucleic acid molecule of Claim 5, wherein the signal peptide is SEQ ID NO:24 or SEQ ID NO:25, and wherein the  $\alpha(1,6)$ -linked glucose oligosaccharide hydrolyzing polypeptide is SEQ ID NOs:2, 4, 6, 17, or 31.

Application No.: 10/648,152

Docket No.: CL2123 US NA

Page 3

9. (original) The isolated nucleic acid molecule of Claim 5, wherein the signal peptide is encoded by the signal sequence as set forth in SEQ ID NO:26 or SEQ ID NO:27.

10. (original) The isolated nucleic acid molecule of Claim 5 encoding the  $\alpha(1,6)$ -linked glucose oligosaccharide hydrolyzing polypeptide, the isolated nucleic acid molecule having the sequence as set forth in SEQ ID NO:1, SEQ ID NO:5, SEQ ID NO:16, or SEQ ID NO:30.

11. (original) The isolated nucleic acid molecule of Claim 5 selected from the group consisting of SEQ ID NO:3, SEQ ID NO:28, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, or SEQ ID NO:42.

12. (withdrawn) The polypeptide encoded by the nucleic acid molecule of Claim 5.

13. (withdrawn) The polypeptide encoded by the isolated nucleic acid molecule of Claim 9, Claim 10, or Claim 11.

14. (withdrawn) The polypeptide of Claim 13 selected from the group consisting of SEQ ID NO:4, SEQ ID NO:29, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, and SEQ ID NO:43.

15. (canceled) A chimeric gene comprising the isolated nucleic acid molecule of Claim 1 or Claim 5 operably linked to suitable regulatory sequences.

16. (canceled) The chimeric gene of Claim 15 wherein the suitable regulatory sequence is selected from the group comprising *CYC1*, *HIS3*, *GAL1*, *GAL10*, *ADH1*, *PGK*, *PHO5*, *GAPDH*, *ADC1*, *TRP1*, *URA3*, *LEU2*, *ENO*, *TPI*, *AOX1*, *lac*, *ara*, *tet*, *trp*, *IP<sub>L</sub>*, *IP<sub>R</sub>*, *T7*, *tac*, *trc*, *apr*, *npr*, *nos*, and *Gl*.

17. (canceled) A vector comprising the chimeric gene of Claim 15.

18. (canceled) A transformed host cell comprising the chimeric gene of Claim 15.

19. (canceled) The transformed host cell of Claim 18 wherein the chimeric gene is integrated into the chromosome or is plasmid-borne.

20. (canceled) The transformed host cell of Claim 18, wherein the host cell is selected from the group consisting of bacteria, yeast, and filamentous fungi.

21. (canceled) The transformed host cell of Claim 20, wherein the transformed host cell is selected from the genera *Aspergillus*, *Trichoderma*, *Saccharomyces*, *Pichia*, *Candida*, *Hansenula*, *Rhodococcus*, *Acinetobacter*, *Arthrobacter*, *Brevibacterium*, *Acidovorax*, *Bacillus*, *Streptomyces*, *Escherichia*, *Salmonella*, *Pseudomonas*, or *Corynebacterium*.

22. (canceled) The transformed host cell of Claim 20 wherein the transformed host cell is *E. coli*.

Application No.: 10/648,152

Docket No.: CL2123 US NA

Page 4

23. (withdrawn) A method for the production of a target molecule in a recombinant host cell comprising:

- (a) contacting a transformed host cell comprising:
  - (i) an isolated nucleic acid molecule encoding a chimeric protein comprised of a signal peptide operably linked to an  $\alpha(1,6)$ -linked glucose oligosaccharide hydrolyzing polypeptide;
  - (ii) a nucleic acid molecule that hybridizes with (i) under the following hybridization conditions: 0.1X SSC, 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS; or
  - (iii) a nucleic acid molecule that is complementary to (i) or (ii); and
  - (iv) at least one chimeric gene for converting monosaccharides to the target molecule,

in the presence of limit dextrin under suitable conditions whereby the target molecule is produced; and

- (b) optionally recovering the target molecule produced in (a).

24. (withdrawn) A method for the production of glycerol in a recombinant host cell comprising:

- (a) contacting a transformed host cell comprising:
  - (i) an isolated nucleic acid molecule encoding a chimeric protein comprised of a signal peptide operably linked to an  $\alpha(1,6)$ -linked glucose oligosaccharide hydrolyzing polypeptide;
  - (ii) a nucleic acid molecule that hybridizes with (i) under the following hybridization conditions: 0.1X SSC, 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS; or
  - (iii) a nucleic acid molecule that is complementary to (i) or (ii); and
  - (iv) at least one chimeric gene for converting monosaccharides to glycerol,

in the presence of limit dextrin under suitable conditions whereby glycerol is produced; and

- (b) optionally recovering the glycerol produced in (a).

25. (withdrawn) A method for the production of 1,3-propanediol in a recombinant host cell comprising:

- (a) contacting a transformed host cell comprising:
  - (i) an isolated nucleic acid molecule encoding a chimeric protein comprised of a signal peptide operably linked to an  $\alpha(1,6)$ -linked glucose oligosaccharide hydrolyzing polypeptide;

Application No.: 10/648,152  
Docket No.: CL2123 US NA

Page 5

- (ii) a nucleic acid molecule that hybridizes with (i) under the following hybridization conditions: 0.1X SSC, 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS; or
- (iii) a nucleic acid molecule that is complementary to (i) or (ii),
- (iv) at least one chimeric gene for converting monosaccharides to 1,3-propanediol,

in the presence of limit dextrin under suitable conditions whereby 1,3-propanediol is produced; and

(b) optionally recovering the 1,3-propanediol produced in (a).

26. (withdrawn) A method for the production of cell mass in a recombinant host cell comprising:

- (a) contacting a transformed host cell comprising:
    - (i) an isolated nucleic acid molecule encoding a chimeric protein comprised of a signal peptide linked to an  $\alpha(1,6)$ -linked glucose oligosaccharide hydrolyzing polypeptide;
    - (ii) a nucleic acid molecule that hybridizes with (i) under the following hybridization conditions: 0.1X SSC, 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS; and
    - (iii) a nucleic acid molecule that is complementary to (i) or (ii).
- under suitable conditions in the presence of limit dextrin;

(b) optionally recovering the cell mass produced in (a).

27. (withdrawn) The method of Claim 23, Claim 24, Claim 25 or Claim 26 wherein the signal peptide comprises SEQ ID NO:24 or SEQ ID NO:25.

28. (withdrawn) A method for the production of a target molecule in a recombinant host cell comprising:

- (a) contacting a transformed host cell comprising:
  - (i) an isolated nucleic acid molecule encoding the amino acid sequence selected from the group consisting of SEQ ID NOs:2, 6, 17 and 31;
  - (ii) a nucleic acid molecule that hybridizes with (i) under the following hybridization conditions: 0.1X SSC, 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS; or
  - (iii) a nucleic acid molecule that is complementary to (i) or (ii); and
  - (iv) at least one chimeric gene for converting monosaccharides to the target molecule,

Application No.: 10/648,152

Docket No.: CL2123 US NA

Page 6

in the presence of limit dextrin under suitable conditions whereby the target molecule is produced; and

(b) optionally recovering the target molecule produced in (a).

29. (withdrawn) A method for the production of 1,3-propanediol in a recombinant host cell comprising:

(a) contacting a transformed host cell comprising:

- (i) an isolated nucleic acid molecule encoding the amino acid sequence selected from the group consisting of SEQ ID NOs:2, 6, 17 and 31;
- (ii) a nucleic acid molecule that hybridizes with (i) under the following hybridization conditions: 0.1X SSC, 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS; or
- (iii) a nucleic acid molecule that is complementary to (i) or (ii); and
- (iv) at least one chimeric gene for converting monosaccharides to 1,3-propanediol;

in the presence of limit dextrin under suitable conditions whereby 1,3-propanediol is produced; and

(b) optionally recovering the 1,3-propanediol produced in (a).

30. (withdrawn) A method for the production of glycerol in a recombinant host cell comprising:

(a) contacting a transformed host cell comprising:

- (i) an isolated nucleic acid molecule encoding the amino acid sequence selected from the group consisting of SEQ NOs:2, 6, 17 and 31;
- (ii) a nucleic acid molecule that hybridizes with (i) under the following hybridization conditions: 0.1X SSC, 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS; or
- (iii) a nucleic acid molecule that is complementary to (i) or (ii); and
- (iv) at least one chimeric gene for converting monosaccharides to glycerol;

in the presence of limit dextrin under suitable conditions whereby glycerol is produced; and

(b) optionally recovering the glycerol produced in (a).

31. (withdrawn) A method for the production of cell mass in a recombinant host cell comprising:

(a) contacting a transformed host cell comprising:

Application No.: 10/648,152  
Docket No.: CL2123 US NA

Page 7

- (i) an isolated nucleic acid molecule encoding the amino acid sequence selected from the group consisting of SEQ ID NOs:2, 6, 17 and 31;
  - (ii) a nucleic acid molecule that hybridizes with (i) under the following hybridization conditions: 0.1X SSC, 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS; or
  - (iii) a nucleic acid molecule that is complementary to (i) or (ii),  
in the presence of limit dextrin under suitable conditions whereby cell mass is produced; and
  - (b) optionally recovering the cell mass produced in (a).
32. (withdrawn) The method of Claim 28, Claim 29, Claim 30 or Claim 31 wherein the signal peptide is SEQ ID NO:24 or SEQ ID NO:25.
33. A method for degrading limit dextrin comprising:
- (a) contacting a transformed host cell comprising:
    - (i) a nucleic acid molecule encoding the enzymes selected from the group consisting of SEQ ID NOs:2, 6, 17 and 31;
    - (ii) a nucleic acid molecule that hybridizes with (i) under the following hybridization conditions: 0.1X SSC, 0.1 % SDS, 65°C and washed with 2X SSC, 0.1 % SDS followed by 0.1X SSC, 0.1 % SDS; or
    - (iii) a nucleic acid molecule that is complementary to (i) or (ii),  
with an effective amount of limit dextrin substrate under suitable growth conditions; and
  - (b) optionally recovering the product of step (a).
34. (withdrawn) A polypeptide having an amino acid sequence that has at least 69% identity based on the BLASTP method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:17, the polypeptide having  $\alpha(1,6)$ -linked glucose oligosaccharide hydrolyzing activity.